REMARKS

The instant application is a divisional application, filed on February 26, 1999 pursuant to 37 C.F.R. §1.53(b), of pending prior application serial number 08/461,598, filed on June 5, 1995 ("the parent application"), now U.S. Patent 5,876,951.

Claims 43-99 were pending in the application. Claims 48, 59, 73, 81, 90, 97 and 99 have been amended, and claims 100-109 have been added. Accordingly, claims 43-109 will be pending upon entry of the claim amendments and additions presented herein.

Claims 48, 59, 73, 81, 90, 97 and 99 have been amended and claims 100-109 have been added to claim more fully the instant invention and/or to correct minor typographical errors. In addition, pursuant to 37 C.F.R. §§ 1.121 (b)(3) and 1.125(b), the specification has been amended by replacing the specification as originally filed with the Substitute Specification submitted herewith.

Support for the claim amendments can be found throughout the specification and claims as originally filed. In particular, support for the amendments to claims 59, 73, 81, and 90 can be found at least, for example, in the specification at page72, lines 8-11. Support for the addition of claim 100-109 can be found at least, for example, in claims 43, 45, 46, 47, 49, 53, 63, 64, 67, 68, 94, 95, 96, 97, 98 and 99 as originally filed, and in the specification at page 76, line 25. Support for the amendments to the specification can be found at least, for example, in the specification and Sequence Listing as originally filed. No new matter has been added.

Attached hereto as Appendix A is a version of the claims with markings to show the changes made. For the Examiner's convenience, a clean set of all the claims that will be pending in the application is attached hereto as Appendix B.

Amendment or cancellation of the claims should in no way be construed as an acquiescence to any of the rejections set forth in the instant Office Action, and was done solely to expedite prosecution. Applicants reserve the right to pursue the claims as originally filed in this or a separate application(s).

Priority

Applicants respectfully acknowledge the Examiner's indication that Applicants' claim for domestic priority has been granted. In item 8 on page 2 of the divisional application transmittal letter that was concurrently filed with the instant application, Applicants requested that the first line of the application be amended as follows:

"This application is a divisional application of serial no. 08/461,598 filed on June 5, 1995 and issued as U.S. Patent 5,876,951 March 2, 1999, which in turn is a continuation-in-part application of serial no. 08/322,137 filed on October 13, 1994, now pending, which is a continuation-in-part of serial no. 08/309,313, filed September 20, 1994, now abandoned, which is a continuation-in-part of serial no. 08/190,328, filed January 31, 1994, now abandoned, which is a continuation-in-part of Serial No. 08/041,431, filed March 31, 1993, now abandoned. The contents of all of the aforementioned application(s) are hereby incorporated by reference."

Applicants respectfully request the Examiner to acknowledge that this amendment to the specification has been made. Applicants also advise the Examiner that U.S. Ser. No. 08/322,137, filed October 13, 1994, referenced in the above paragraph, has since issued as U.S. Patent 6,100,042 on August 8, 2000. Applicants respectfully request the Examiner to update the priority information accordingly.

Objection to the Drawings

The Office Action indicates that new corrected drawings are required based on the reasons set forth in the Draftsperson's comments in form PTO-948.

Applicants submit herewith corrected drawings, and respectfully request reconsideration and withdrawal of the objection to the drawings.

Objection to the Specification

The specification is objected to because the specification contains multiple peptide sequences greater than 4 amino acids in length, requiring that they be identified by SEQ ID NOS.

Applicants submit herewith a computer readable form of a substitute Sequence Listing and a hard (paper) copy of the substitute Sequence Listing, both of which include all of the sequences that are present in the application. The content of the hard copy of the substitute Sequence Listing and the computer readable form of the substitute Sequence Listing are the same

and include no new matter. In addition, an amendment directing entry of the hard copy of the substitute Sequence Listing into the specification is set forth above. Further, the specification has been amended to insert sequence identifiers. A substitute specification is submitted herewith in compliance with 37 C.F.R. § 1.125(b), in addition to a marked up version of the substitute specification showing the changes made. Accordingly, Applicants respectfully request that the objections pertaining to the specification be withdrawn.

Claim Objections

The Office Action, at page 3, indicates that claim 59 is objected to because the claim recites the terminology 'an IGP dehydratase gene' without first identifying what the abbreviation IGP represents.

In accordance with the Examiner's helpful suggestion, Applicants have amended claim 59 to recite the genetic name for this gene (*HIS3*). Therefore, Applicants respectfully request reconsideration and withdrawal of the objection to claim 59.

The Office Action, at page 4, also notes that the "yeast genes (and proteins; for example with respect to Ste3 in claim 97) all have a space between the letters and the numbers and that this does not conform to proper nomenclature."

Applicants have amended the relevant claims to recite the proper nomenclature. Therefore, Applicants respectfully request reconsideration and withdrawal of the objection to the claims.

Claim Rejections Under 35 U.S.C. §112, Second Paragraph

Claim 48 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. In particular, the Office Action asserts that "the term 'endogenous pheromone system protein' lacks antecedent basis and is indefinite because it is unclear if the claim is

referring to any pheromone system protein, or a specific pheromone system protein (*e.g.* the endogenous protein corresponding to the first heterologous gene)."

Applicants have amended claim 48 to recite the proper antecedent basis. Therefore, Applicants respectfully request reconsideration and withdrawal of the rejection.

Claim Rejections Under 35 U.S.C. §102

Claims 43, 48, 54, 57, 58, 60, 61, 63-65, 96, and 97 are rejected under 35 U.S.C. §102(e) as being anticipated by King *et al.* (U.S. Patent No. 5,482,835; hereinafter King). Applicants respectfully traverse and submit that King does not anticipate the claimed invention.

The Examiner indicates that the claims are anticipated because King teaches "a yeast cell having a pheromone system, where the yeast cell co-expresses a first hetereologous gene encoding a surrogate of a yeast pheromone receptor (βAR) and a second hetereologous gene encoding a peptide (Gαs) that modulates the interaction of the receptor with the pheromone system." The Examiner further indicates that King teaches that the yeast cell can either be desensitized to the pheromone signaling cascade upon the deletion of the endogenous Gα subunit, encoded by the *GPA1* gene or it can be wild type with respect to the pheromone signaling cascade.

Pending claims 43-99 are directed to a yeast cell comprising a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor and a second heterologous gene encoding a heterologous peptide which modulates the interaction of the surrogate with the pheromone system. The salient feature of the claimed invention is the coexpression of a pheromone system surrogate and a heterologous peptide that ***modulates***, *i.e.*, functionally interacts with the pheromone system protein surrogate, thereby generating intracellular signals that are capable of being screened or selected for.

The King *et al.* patent teaches a transformed yeast cell comprising a first heterologous DNA sequence that codes for a mammalian G protein coupled receptor (*e.g.*, βAR) and a second heterologous DNA sequence that codes for a mammalian Gα (*e.g.*, Gαs), wherein the cell expresses Gβγ (endogenous yeast G protein subunits), and wherein the mammalian Gα and the Gβγ can complex to form a functional G protein. The patent also teaches methods using these

transformed yeast cells to test compounds for the ability to affect the rate of dissociation of Gα from Gβγ in the transformed yeast cells. The ability of the test compound to affect the rate of dissociation of Gα from Gβγ is indicative of the compound's ability to interact with the mammalian G protein coupled receptor, and to thereby modulate (upregulate or downregulate) the interaction of the receptor with the yeast pheromone system.

Applicants submit that King neither teaches nor suggests a second heterologous gene that encodes a heterologous peptide that modulates the interaction of the surrogate with the yeast pheromone system. Although the mammalian Gα (*e.g.*, Gαs) of the King patent is a heterologous polypeptide with respect to the transformed yeast cell, it is a G protein subunit that complexes with the Gβγ subunits to form a functional G protein, and, therefore, does not modulate (as that term is understood in the art) the interaction of the receptor with pheromone system.

King *et al.* selected a mammalian Gα subunit because they discovered that the mammalian receptor would not functionally couple with the yeast G protein; *i.e.*, a yeast cell transformed only with heterologous DNA that expressed βAR was not responsive to adrenergic ligands. They discovered that when the yeast cell is engineered to express a mammalian Gα subunit and not to express the endogenous yeast Gα subunit, the mammalian receptor readily coupled with the hybrid G protein. In other words, King *et al.* expressed the heterologous Gα subunit in order to achieve functional coupling of the heterologous receptor with the G protein. (See column 8, Example 4). Without functional coupling, the assay system disclosed in the patent would not be able to test the ability of test compounds to affect the rate of dissociation of Gα from Gβγ.

Therefore, one of ordinary skill in the art would understand that the mammalian Gα subunit ***mediates*** the interaction between the mammalian receptor and the G protein, but would not consider that the mammalian Gα subunit ***modulates*** (upregulates or downregulates) the interaction of the receptor and the yeast pheromone system as recited in the instant claims. Mediation and modulation are two different phenomena, and without mediation between the receptor and the G protein to bring about functional coupling, there could be no modulation of the pheromone by interaction of a compound with the receptor. The mammalian Gα subunit of King *et al.* neither upregulates (agonizes) nor downregulates (antagonizes) the pheromone system. Rather, it merely mediates the interaction between the receptor and the G protein so that functional coupling is achieved, and upregulation or downregulation can occur and be measured.

Furthermore, the assay system disclosed in the King patent is limited to detecting the effects (upregulation or downregulation) of exogenously added compounds on G protein coupled receptors by detecting the dissociation of $G\alpha$ from $G\beta\gamma$. The patent does not teach or suggest the "autocrine" system of the instant invention, in which the yeast cell itself expresses the modulator of the pheromone system protein surrogate (*e.g.*, the receptor).

Because the King *et al.* patent does not teach each and every element of the invention as claimed herein, it cannot be said to anticipate the claims. Accordingly, Applicants respectfully request that the rejection of claims 43, 48, 54, 57, 58, 60, 61, 63-65, 96, and 97 under 35 U.S.C. §102(e) be reconsidered and withdrawn.

Applicants further submit that new claims 100-110 are patentable over the King patent under 35 U.S.C. §102(e).

Claim Rejections Under 35 U.S.C. §103

Claim 55 is rejected under 35 U.S.C. 103(a) as being unpatentable over King as applied above in view of Reneke *et al.* (*Cell* 55:226-234, 1988; hereinafter Reneke). Applicants respectfully traverse the rejection and submit that the Office Action fails to set forth a *prima facie* showing of obviousness.

The Office Action alleges that it would have been obvious to the ordinary skilled artisan to combine the teachings of King and Reneke because both references involve the activation of the pheromone signaling pathway in yeast, hence the teachings are related to the same technical field. Further motivation is allegedly provided in the disclosure of Reneke, namely, that *SST2* is involved in the desensitization of a yeast host cell to activation of the pheromone response pathway, and that inactivation of the *SST2* gene results in an increased sensitivity of the yeast host cell to the pheromone response pathway, which results in a greater ability to detect activation of a pheromone response. It is further alleged that one of ordinary skill in the art would have been motivated to combine these teachings in order to accentuate the level of detection of the pheromone response in King because the inactivation of SST2 clearly would enhance the response to pheromone receptor activation, as taught by Reneke. The Office Action concludes that "absent evidence to the contrary and given the teachings of the stated prior art and the high level of skill of the ordinary skilled artisan at the time of the Applicants' invention, it

must be considered that said artisan would have had a reasonable expectation of success in practicing the claimed invention."

Applicants respectfully disagree and assert that the cited combination of references fails to establish a *prima facie* showing of obviousness. To establish a *prima facie* case of obviousness there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, the prior art reference (or references when combined) must teach or suggest all the claim limitations (that is, put one of ordinary skill in the art in possession of the invention). Finally, there must be a reasonable expectation of success. See M.P.E.P. §2143. Applicants submit that the claimed invention is nonobvious over the art of record because, at a minimum, one of ordinary skill in the art would have no motivation to combine the references in the manner suggested in the Office Action, and even if one were to so combine the cited references, there would be no reasonable expectation of success.

Claim 55, depending from claims 54 and 43, recites a yeast cell comprising (a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, the surrogate performing in the pheromone system of the yeast cell a function naturally performed by the yeast pheromone receptor, and (b) a second heterologous gene encoding a heterologous peptide, wherein the heterologous peptide modulates the interaction of the surrogate with the pheromone system in the yeast cell, and the modulation is a selectable or screenable event, and wherein the cell is a mutant strain having a pheromone signal pathway that is desensitized at slower rate relative to the wild type strain under the same conditions of continuous stimulation of the pheromone signal pathway and wherein the endogenous *SST2* gene is not functionally expressed.

Applicants submit that none of the cited references, alone or in combination, teaches or suggests the claimed invention. The King *et al.* patent was discussed at length above and that discussion is reiterated here. In particular, the King *et al.* patent neither teaches nor suggests a second heterologous gene encoding a heterologous peptide that ***modulates*** the interaction of the surrogate with the pheromone system. As noted above, King teaches the expression of a heterologous mammalian Gα subunit that ***mediates*** the interaction between the mammalian receptor and the G protein. However, the mammalian Gα does not ***modulate*** (upregulate or downregulate) the interaction of the receptor and the yeast pheromone system as recited in the

instant claims. It simply allows functional interaction of the receptor and the G protein so that modulation of the pheromone system by ligands (*e.g.*, antagonists or agonists) can occur and be measured. Moreover, one of ordinary skill in the art would readily appreciate that mediation of the interaction between the receptor and the G protein is not the same phenomenon as modulation of the interaction of the receptor with the pheromone system.

The Examiner correctly notes that King does not teach the further disruption of SST2 to prevent desensitization of the host cell to the activation of the pheromone signaling pathway. As set forth above, it is Applicants' position that the teachings of King are limited to the expression of a heterologous G protein coupled receptor and a heterologous Gα subunit in yeast cells. There is no teaching or suggestion in King to inactivate an endogenous gene to increase the sensitivity of the assay, nor is there any teaching or suggestion that such inactivation would even be desirable. As noted above, the motivation to combine must be present in the teachings of the reference, and the motivation cannot be derived from the teachings of the application under examination. Given the clear absence in King of any teaching or suggestion to inactivate an endogenous gene to increase the sensitivity of the assay, the position taken by the Examiner is nothing more than an impermissible hindsight reconstruction of the invention ***based on Applicants' own teachings***.

Reneke teaches that a mutant SST2 gene causes yeast cells carrying the mutation to be significantly more sensitive to pheromone than wild type yeast cells. It is important to note that Reneke *et al.* discovered that complete removal of the C-terminal domain of the endogenous yeast STE2 receptor did not eliminate its ability to bind α-factor and to promote mating; hence the recognition function and signal transmission functions of the receptor do not require the C-terminal residues of the receptor. (Reneke *et al.*, page 228, left column, first full paragraph). The endogenous ligand-binding and signal transmission functions of the STE2 receptor remained unchanged such that, notwithstanding the truncation of or complete removal of the C-terminus of the receptor, the mutant would not be considered a heterologous surrogate because its natural function has been unaltered and, therefore, requires no surrogate for that function.

Thus, the reference neither teaches nor suggests a first heterologous gene encoding ***a heterologous surrogate*** of a yeast pheromone receptor that performs in the pheromone system of the yeast cell a function naturally performed by the yeast pheromone receptor. Likewise, the reference is devoid of any teaching or suggestion of ***a second heterologous gene encoding a heterologous peptide***, wherein the heterologous peptide modulates the interaction of the

surrogate with the pheromone system in the yeast cell, or modulation as a selectable or screenable event. The reference merely discloses use of the natural ligand (α-factor) for the receptor.

Thus, the Reneke *et al.* reference does not make up for the deficiencies of the King *et al.* patent. Unlike the King patent, the reference does not teach or suggest the use of transformed yeast cells in an assay to screen for compounds that affect the rate of dissociation of Gα from G$\beta\gamma$ in the cell (*i.e.*, modulate the pheromone system). Consequently, one of ordinary skill in the art would not be motivated to combine the reference with the King patent. Moreover, assuming there were some motivation to combine the references, the combination would not provide all the elements of claim 55 because the second heterologous gene encoding a heterologous peptide that modulates the interaction of the surrogate with the pheromone system would still be lacking. In the absence of this element, the combination of references does not put one of ordinary skill in possession of the invention as claimed, and, consequently, there could be no reasonable expectation of success.

In summary, Applicants assert that the art of record fails to provide the motivation to combine the references in the manner suggested by the Examiner to make the claimed invention, that a combination of the art of record would not put the artisan or ordinary skill in possession of the invention, nor would it provide the artisan with a reasonable expectation of success in making the claimed invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claim 55 under 35 U.S.C. §103(a).

Rejection of Claim 56 Under 35 U.S.C. § 103 (a)

Claim 56 is rejected under 35 U.S.C. 103(a) as being unpatentable over King as applied above in view of Chang *et al.* (*Cell* 63:999-1011, 1990; hereinafter Chang). Applicants disagree and respectfully traverse the rejection.

The Examiner's analysis of the King *et al.* reference as applied to this rejection is the same as that for the rejection of claim 55, and is set forth above. The Examiner further indicates that Chang teaches that the *FAR1* gene product is necessary for cell cycle arrest of yeast cells that are responding to activation of the pheromone pathway, and that mutating the *FAR1* gene results in the activation of the pheromone signaling pathway in the absence of cell cycle arrest. The Examiner concludes that one of ordinary skill in the art would be motivated to combine the references because "both references involve the activation of the pheromone signaling pathway

in yeast." And "to prevent the host cells of King from experiencing a growth arrest in response to the activation of the surrogate pheromone signaling pathway, therefore avoiding a potential impediment to the detection assay comprising cell growth arrest and a corresponding drop in signal detection, as taught by Chang."

Applicants respectfully disagree. Claim 56, depending from claims 43, recites a yeast cell comprising (a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, the surrogate performing in the pheromone system of the yeast cell a function naturally performed by the yeast pheromone receptor, and (b) a second heterologous gene encoding a heterologous peptide, wherein the heterologous peptide modulates the interaction of the surrogate with the pheromone system in the yeast cell, and the modulation is a selectable or screenable event, and in which the endogenous *FAR1* gene is not functionally expressed.

Applicants have distinguished King above, and reiterate those comments here. The Examiner correctly notes that King does not teach the further disruption of *FAR1* to prevent cell growth arrest in the host cell following activation of the pheromone signaling pathway. Applicants further submit that there is no teaching or suggestion in King to inactivate an endogenous gene (the *FAR1* gene) to prevent cell cycle arrest from impeding the assay, nor is there any teaching or suggestion that such inactivation would even be desirable. As noted above, the motivation to combine must be present in the teachings of the reference, and the motivation cannot be derived from the teachings of the application under examination. Given the clear absence in King of any teaching or suggestion to inactivate an endogenous gene to prevent cell cycle arrest from impeding the assay, the position taken by the Examiner is nothing more than an impermissible hindsight reconstruction of the invention ***based on Applicants' own teachings***.

Chang teaches that yeast cells carrying the *FAR1* gene mutation were unable to arrest in response to α-factor, in contrast to wild-type yeast cells. However, the reference neither teaches nor suggests a first heterologous gene encoding ***a heterologous surrogate*** of a yeast pheromone receptor that performs in the pheromone system of the yeast cell a function naturally performed by the yeast pheromone receptor. Likewise, the reference is devoid of any teaching or suggestion of ***a second heterologous gene encoding a heterologous peptide***, wherein the heterologous peptide modulates the interaction of the surrogate with the pheromone system in the yeast cell, or modulation as a selectable or screenable event. The reference merely discloses

use of the endogenous yeast receptors (STE2 or STE3) and the natural ligands (α-factor or a-factor) for the receptors.

Thus, the Change *et al.* reference does not make up for the deficiencies of the King *et al.* patent. Unlike the King patent, the reference does not teach or suggest the use of transformed yeast cells in an assay to screen for compounds that affect the rate of dissociation of Gα from Gβγ in the cell (*i.e.*, modulate the pheromone system). Consequently, one of ordinary skill in the art would not be motivated to combine the reference with the King patent. Moreover, assuming there were some motivation to combine the references, the combination would not provide all the elements of claim 56 because the second heterologous gene encoding a heterologous peptide that modulates the interaction of the surrogate with the pheromone system would still be lacking. In the absence of this element, the combination of references does not put one of ordinary skill in possession of the invention as claimed, and, consequently, there could be no reasonable expectation of success.

In summary, Applicants assert that the art of record fails to provide the motivation to combine the references in the manner suggested by the Examiner to make the claimed invention, that a combination of the art of record would not put the artisan or ordinary skill in possession of the invention, nor would it provide the artisan with a reasonable expectation of success in making the claimed invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claim 56 under 35 U.S.C. §103(a).

Rejection of Claim 59 Under 35 U.S.C. § 103 (a)

Claim 59 is rejected under 35 U.S.C. 103(a) as being unpatentable over King as applied above in view of Sikorski *et al.* (*Genetics* 122: 19-27, 1989; hereinafter Sikorski). Applicants disagree and respectfully traverse the rejection.

The Examiner's analysis of the King *et al.* reference as applied to this rejection is the same as that for the rejection of claim 59, and is set forth above. The Examiner asserts that Sikorski teaches the use of *HIS3* as a selectable marker for the indication of transcriptional activation in autotrophic mutations of *HIS3* as a means for screening yeast cells, allowing for a nutritional selection without the need for an additional assay to determine the presence of the HIS gene. The Examiner concludes that it would have been obvious to the ordinary skilled artisan to combine the teachings of the referenced because "both references correspond to

selection of a particular yeast that expresses a genetic marker, although the genetic markers are different (King uses LacZ whereas Sikorski uses *HIS3*)". The Examiner further contends that the artisan "would have been motivated to combine the teachings because the method of Sikorski involves a nutritional selection and does not require an enzymatic assay, such as the β-galactosidase assay required for monitoring the presence of lacZ in King, therefore the selection process would be simpler and more rapid."

Applicants respectfully disagree. Claim 59, depending from claims 59, 58, 57 and 43, recites a yeast cell comprising (a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, the surrogate performing in the pheromone system of the yeast cell a function naturally performed by the yeast pheromone receptor, (b) a second heterologous gene encoding a heterologous peptide, wherein the heterologous peptide modulates the interaction of the surrogate with the pheromone system in the yeast cell, and the modulation is a selectable or screenable event, (c) a selectable marker that is activated by the pheromone signal pathway, wherein the selectable marker comprises a pheromone-responsive promoter which is substantially homologous with an endogenous pheromone-responsive promoter, operably linked to a foreign selectable gene which is an *HIS3* gene.

Applicants have distinguished King above, and reiterate those comments here. The Examiner correctly notes that King does not teach the use of *HIS3* as a selectable marker for the indication of transcriptional activation.

Applicants further submit that there is no teaching or suggestion in King to use *HIS3* as a selectable marker for the indication of transcriptional activation, nor is there any teaching or suggestion that such use would even be desirable. As noted above, the motivation to combine must be present in the teachings of the reference, and the motivation cannot be derived from the teachings of the application under examination. Given the clear absence in King of any teaching or suggestion to the use of *HIS3* as a selectable marker for the indication of transcriptional activation, the position taken by the Examiner is nothing more than an impermissible hindsight reconstruction of the invention ***based on Applicants' own teachings***.

Sikorski discloses a series of yeast shuttle vectors and host strains that were created to allow for more efficient manipulation of DNA in *S. cerevisiae*. The reference discloses the construction and use of transplacement vectors to derive yeast strains containing four nonreverting mutations, including a *his3* mutation, thereby providing four yeast selectable markers.

However, the reference neither teaches nor suggests a first heterologous gene encoding ***a heterologous surrogate*** of a yeast pheromone receptor that performs in the pheromone system of the yeast cell a function naturally performed by the yeast pheromone receptor. Likewise, the reference is devoid of any teaching or suggestion of ***a second heterologous gene encoding a heterologous peptide***, wherein the heterologous peptide modulates the interaction of the surrogate with the pheromone system in the yeast cell, or modulation as a selectable or screenable event.

Thus, the Sikorski *et al.* reference does not make up for the deficiencies of the King *et al.* patent. Unlike the King patent, the reference does not teach or suggest the use of transformed yeast cells in an assay to screen for compounds that affect the rate of dissociation of $G\alpha$ from $G\beta\gamma$ in the cell (*i.e.*, modulate the pheromone system). Consequently, one of ordinary skill in the art would not be motivated to combine the reference with the King patent. In fact, if, according to the Examiner, the method of Sikorski involves a nutritional selection and does not require an enzymatic assay, such as the β-galactosidase assay required for monitoring the presence of lacZ in King, such the selection process would be simpler and more rapid, then the Sikorski reference constitutes a teaching away from the King *et al.* patent, such that one of ordinary skill in the art would not be motivated to look to the teachings of the King patent.

Moreover, assuming there were some motivation to combine the references, the combination would not provide all the elements of claim 59 because the second heterologous gene encoding a heterologous peptide that modulates the interaction of the surrogate with the pheromone system would still be lacking. In the absence of this element, the combination of references does not put one of ordinary skill in possession of the invention as claimed, and, consequently, there could be no reasonable expectation of success.

In summary, Applicants assert that the art of record fails to provide the motivation to combine the references in the manner suggested by the Examiner to make the claimed invention, that a combination of the art of record would not put the artisan or ordinary skill in possession of the invention, nor would it provide the artisan with a reasonable expectation of success in making the claimed invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claim 56 under 35 U.S.C. §103(a).

Double Patenting

Claims 43, 46, 48-52, 54-66, and 69-93 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-4, 6-18, 24, 29, 35, 36, 38, 39, and 41 of U.S. Patent No. 6,100,042. Applicants will address this rejection at such time as the claims are indicated as being allowable, but for the obviousness-type double patenting rejection.

CONCLUSION

In view of the foregoing amendments and arguments, Applicants submit that the rejections of the pending claims have been overcome thereby putting the claims in condition for allowance. Accordingly, entry of the foregoing amendments and remarks, reconsideration and withdrawal of all rejections, and allowance of all pending claims are respectfully requested.

If a telephone conversation with Applicants' Attorney would expedite the prosecution of the above-identified application, the examiner is urged to call Applicants' Attorney at (617) 227-7400.

Respectfully submitted,

Hathaway Pease, Esq.
Registration No. 46,488
Attorney for Applicants

LAHIVE & COCKFIELD, LLP
28 State Street
Boston, MA 02109
Tel. (617) 227-7400

Dated: May 14, 2003

APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

48. **(Currently amended)** The yeast cell of claim 43 wherein said yeast cell comprises an ~~the~~ endogenous pheromone system protein, wherein said protein is not produced in functional form.

59. **(Currently amended)** The yeast cell of claim 58 wherein the selectable gene is an ~~IGP dehydratase~~ *HIS3* gene.

73. **(Currently amended)** The recombinant cells of claim 71, wherein the marker gene that gives rise to a detectable signal is a *HIS3* gene.

81. **(Currently amended)** The recombinant cells of claim 79, wherein the marker gene that gives rise to a detectable signal is a *HIS3* gene.

90. **(Currently amended)** The method of claim 88, wherein the marker gene that gives rise to a detectable signal is a *HIS3* gene.

97. **(Currently amended)** The yeast cell of claim 96, wherein the yeast cell expresses Ste3p.

99. **(Currently amended)** The yeast cell of claim 97, wherein the yeast cell expresses Gal1 under the control of a pheromone responsive promoter and further comprises a mutated form of Gal7 or Gal10.

APPENDIX B

43. A yeast cell having a pheromone system, which cell comprises
(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor, and
(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event.

44. The yeast cell of claim 43 in which the peptide is an agonist for the surrogate receptor.

45. The yeast cell of claim 43 in which the peptide is an antagonist for the surrogate receptor.

46. The yeast cell of claim 43, which further comprises a G protein, said G protein comprising a Gα subunit, wherein said Gα subunit is chimeric.

47. The yeast cell of claim 46 wherein the amino terminal portion of the Gα subunit is substantially homologous with the Gα subunit of a yeast G protein and the remainder is substantially homologous with the corresponding portion of a Gα subunit of a heterologous G protein.

48. The yeast cell of claim 43 wherein said yeast cell comprises an ~~the~~ endogenous pheromone system protein, wherein said protein is not produced in functional form.

49. The yeast cell of claim 43 wherein the heterologous peptide is secreted by the cell into the periplasmic space, from which it interacts with said surrogate.

50. The yeast cell of claim 49, wherein the heterologous peptide is expressed in the form of a precursor peptide comprising a cleavable leader peptide and a mature peptide, which leader peptide directs secretion of said heterologous peptide.

51. The yeast cell of claim 50 wherein the leader peptide corresponds to a leader peptide of the *Saccharomyces cerevisiae* α factor or **a**-factor.

52. The yeast cell of claim 43 in which a wild-type pheromone of the yeast pheromone system is not secreted.

53. The yeast cell of claim 49 wherein the heterologous peptide is also expressed in a nonsecretory form.

54. The yeast cell of claim 43, wherein the cell is a mutant strain having a pheromone signal pathway that is desensitized at slower rate relative to the wild type strain under the same conditions of continuous stimulation of the pheromone signal pathway.

55. The yeast cell of claim 54 in which the endogenous *SST2* gene is not functionally expressed.

56. The yeast cell of claim 43, in which the endogenous *FAR1* gene is not functionally expressed.

57. The yeast cell of claim 43, further comprising a selectable marker that is activated by the pheromone signal pathway.

58. The yeast cell of claim 57, said selectable marker comprising a pheromone-responsive promoter which is substantially homologous with an endogenous pheromone-responsive promoter, operably linked to a foreign selectable gene.

59. The yeast cell of claim 58 wherein the selectable gene is an *HIS3* gene.

60. The yeast cell of claim 58 wherein the homologous wild-type promoter is the *FUS1* promoter.

61. The yeast cell of claim 43 wherein the cells belong to the species *Saccharomyces cerevisiae*.

62. A yeast culture comprising a plurality of yeast cells according to claim 43, said yeast cells collectively expressing a peptide library.

63. A method of assaying a peptide for modulation of the activity of a non-yeast surrogate for a pheromone system protein which comprises providing yeast cells according to claim 43, which cells functionally express said heterologous surrogate and said heterologous peptide, and determining by detecting a change in said selectable or screenable event whether the pheromone signal pathway is activated or inhibited by the interaction of said surrogate and said peptide.

64. The method of claim 63 in which the cells comprise a pheromone-responsive selectable marker, and cells are selected for expression of a peptide having the desired activating or inhibiting effect.

65. The method of claim 63 in which the cells comprise a pheromone-responsive screenable marker, and cells are screened for expression of a peptide having the desired activating or inhibiting effect.

66. A method of assaying a peptide library for activity of a non-yeast pheromone system protein surrogate which comprises providing a yeast culture according to claim 62, whose cells each functionally express said surrogate and a peptide of said library, said culture collectively expressing the entire peptide library, and determining whether the pheromone signal pathway is activated or inhibited by said peptides in each of the cells of said culture.

67. The yeast cell of claim 43 wherein said surrogate is the C5a receptor.

68. The method of claim 64 in which the surrogate is human Mdr1, the cells grow on histidine-free media only if the surrogate transports α-factor, the cells are galactose-sensitive

only if the surrogate transports α-factor, and endogenous pleiotropic drug resistance genes have been inactivated.

69. A mixture of recombinant yeast cells, each cell of which comprises:

(i) a pheromone system generating a detectable signal;

(ii) an expressible gene construct encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor; and

(iii) an expressible gene construct encoding a heterologous peptide,
wherein collectively the mixture of cells express a library of said heterologous peptides, and modulation of the pheromone system by the heterologous peptide provides the detectable signal.

70. The recombinant cells of claim 69, wherein the yeast pheromone receptor is inactivated.

71. The recombinant cells of claim 69, wherein each cell further comprises a marker gene construct containing a marker gene in operative linkage with one or more transcriptional regulatory elements responsive to the pheromone system, expression of the marker gene providing the detectable signal.

72. The recombinant cells of claim 71, wherein the marker gene that gives rise to a detectable signal selected from the group consisting of: β galactosidase, alkaline phosphatase, horseradish peroxidase, exoglucanase, luciferase, and chloramphenicol acetyl transferase.

73. The recombinant cells of claim 71, wherein the marker gene that gives rise to a detectable signal is a *HIS3* gene.

74. The recombinant cells of claim 69, wherein the population of heterologous peptides includes at least 10^3 different peptide sequences.

75. The recombinant cells of claim 69, wherein the population of heterologous peptides includes at least 10^7 different peptide sequences.

76. The recombinant cells of claim 69, wherein the yeast cell is a *Saccharomyces* cell.

77. A mixture of recombinant yeast cells, each cell of which comprises:
(i) a pheromone system generating a detectable signal;
(ii) an expressible gene construct encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor; and
(iii) an expressible gene construct encoding a heterologous peptide, said heterologous peptide including a signal sequence for secretion into the periplasmic space, wherein collectively the mixture of cells express a library of said heterologous peptides, and modulation of the pheromone system by the heterologous peptide provides the detectable signal.

78. The recombinant cells of claim 77, wherein the yeast pheromone receptor is inactivated.

79. The recombinant cells of claim 77, wherein each cell further comprises a marker gene construct containing a marker gene in operative linkage with one or more transcriptional regulatory elements responsive to the pheromone system, expression of the marker gene providing the detectable signal.

80. The recombinant cells of claim 79, wherein the marker gene encodes a gene product that gives rise to a detectable signal selected from the group consisting of: β-galactosidase, alkaline phosphatase, horseradish peroxidase, exo glucanase, luciferase, and chloramphenicol acetyl transferase.

81. The recombinant cells of claim 79, wherein the marker gene that gives rise to a detectable signal is a *HIS3* gene.

82. The recombinant cells of claim 77, wherein the population of heterologous peptides includes at least 10^3 different peptide sequences.

83. The recombinant cells of claim 77, wherein the population of heterologous peptides includes at least 10^7 different peptide sequences.

84. The recombinant cells of claim 77, wherein the yeast cell is a *Saccharomyces* cell.

85. A method for identifying potential effectors of a yeast pheromone surrogate, comprising:

(i) providing a mixture of recombinant yeast cells, each cell of which comprises

(a) a pheromone system generating a detectable signal;

(b) an expressible gene construct encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor; and

(c) an expressible gene construct encoding a heterologous peptide,

wherein collectively the mixture of cells express a library of said heterologous peptides, and modulation of the pheromone system by the heterologous peptide provides the detectable signal; and

(ii) isolating cells from the mixture which exhibit the detection signal.

86. The method of claim 85, wherein the yeast pheromone receptor is inactivated.

87. The method of claim 85, wherein said heterologous peptide includes a signal sequence for secretion into the periplasmic space.

88. The method of claim 85, wherein each cell of the mixture further comprises a marker gene construct containing a marker gene in operative linkage with one or more transcriptional regulatory elements responsive to the signal transduction activity of the cell surface receptor protein, and wherein expression of the marker gene provides the detection signal.

89. The method of claim 88, wherein the marker gene encodes a gene product that gives rise to a detection signal selected from the group consisting of: β galactosidase, alkaline phosphatase, horseradish peroxidase, exo glucanase, luciferase, and chloramphenicol acetyl transferase.

90. The method of claim 88, wherein the marker gene that gives rise to a detectable signal is a *HIS3* gene.

91. The method of claim 85, wherein the population of heterologous peptides includes at least 10^3 different peptide sequences.

92. The method of claim 85, wherein the population of heterologous peptides includes at least 10^7 different peptide sequences.

93. The method of claim 85, wherein the yeast cell is a *Saccharomyces* cell.

94. The yeast cell of claim 43, wherein the yeast cell lacks ras function in the presence of cAMP.

95. The yeast cell of claim 94, wherein the yeast cell comprises a cam mutation.

96. The yeast cell of claim 43 wherein the yeast cell responds to **a** factor.

97. The yeast cell of claim 96, wherein the yeast cell expresses Ste3p.

98. The yeast cell of claim 43, wherein the yeast cell responds to **a** factor and fails to grow on galactose.

99. The yeast cell of claim 97, wherein the yeast cell expresses Gal1 under the control of a pheromone responsive promoter and further comprises a mutated form of Gal7 or Gal10.

100. A yeast cell having a pheromone system, which cell comprises:

(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor, and

(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event, and wherein said heterologous polypeptide is selected from the group consisting of agonists for the surrogate receptor and antagonists of the surrogate receptor.

101. A yeast cell having a pheromone system, which cell comprises:

(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor;

(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event, and wherein said heterologous polypeptide is selected from the group consisting of agonists for the surrogate receptor and antagonists of the surrogate receptor; and

(c) chimeric Gα subunit, wherein the amino terminal portion of the Gα subunit is substantially homologous with the Gα subunit of a yeast G protein and the remainder is substantially homologous with the corresponding portion of a Gα subunit of a heterologous G protein.

102. A yeast cell having a pheromone system, which cell comprises:

(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor, and

(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event, and wherein (i) said heterologous polypeptide is secreted by the cell into the periplasmic

space, from which it interacts with said surrogate, or (ii) is expressed in nonsecretory form.

103. A yeast cell having a pheromone system, which cell comprises:

(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor, wherein said surrogate is a C5a receptor; and

(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event.

104. A method of assaying a peptide for modulation of the activity of a non-yeast surrogate for a pheromone system protein which comprises:

(a) providing yeast cells according to claim 43, which cells functionally express said heterologous surrogate and said heterologous peptide, and in which endogenous pleiotropic drug resistance genes have been inactivated; and

(b) determining by detecting a change in said selectable or screenable event whether the pheromone signal pathway is activated or inhibited by the interaction of said surrogate and said peptide, wherein the surrogate is human Mdr1, and said cells:

(i) comprise a pheromone-responsive selectable marker;

(ii) are selected for expression of a peptide having the desired activating or inhibiting effect;

(iii) grow on histidine-free media only if the surrogate transports α-factor; and

(iv) are galactose-sensitive only if the surrogate transports α-factor.

105. A yeast cell having a pheromone system, which cell comprises:

(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor, and

(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event; and wherein the yeast cell lacks ras function in the presence of cAMP.

106. The yeast cell of claim 105, wherein the yeast cell comprises a cam mutation.

107. A yeast cell having a pheromone system, which cell comprises:
(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor, and
(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event; and wherein the yeast cell responds to a factor and fails to grow on galactose.

108. A yeast cell having a pheromone system, which cell comprises:
(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor, and
(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event; and wherein the yeast cell:

(i) responds to **a** factor;

(ii) expresses Ste3p; and

(iii) expresses Gal1 under the control of a pheromone responsive promoter and further comprises a mutated form of Gal7 or Gal10.

109. A yeast cell having a pheromone system, which cell comprises

(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor, and

(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event, and wherein the heterologous peptide is 2 to 200 amino acids in length.